Serum and Synovial Fluid Concentrations of Matrix Metalloproteinases 3 and Its Tissue Inhibitor 1 in Juvenile Idiopathic Arthritides

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ABSTRACT. ***Objective***. Matrix metalloproteinases (MMP) are a large family of proteolytic enzymes involved in the remodeling of extracellular matrix during tissue resorption in idiopathic arthritides. We investigated serum and synovial fluid (SF) concentrations of MMP-3 and its tissue inhibitor (TIMP-1) in juvenile idiopathic arthritides (JIA).

Methods. Sera from 45 patients with active, 15 patients with inactive JIA, and 15 healthy controls were evaluated by ELISA for MMP-3 (stromelysin-1), TIMP-1, and soluble p75 tumor necrosis factor receptor (sTNFR). Paired SF concentrations were evaluated in 19 patients with JIA.

Results. MMP-3 serum concentrations were significantly higher in patients with active poly- and oligoarticular JIA versus inactive patients ($p = 0.04$ and $p = 0.02$, respectively) and healthy controls ($p < 0.001$ for both). Serum MMP-3, but not TIMP-1, concentration displayed a variable degree of correlation with clinical and laboratory variables of disease activity and with p75 sTNFR concentrations ($r = 0.37$, $p = 0.005$). SF MMP-3 concentrations were 30–300 times higher than those found in paired sera ($p < 0.001$, Wilcoxon rank test). A clear inversion of MMP-3/TIMP-1 ratio was observed when sera (median 0.31, range 0.02–1.5) were compared with the corresponding SF samples (5.3, range 4.9–5.5; $p < 0.001$).

Conclusion. MMP-3 (stromelysin-1) is clearly overexpressed in SF of patients with JIA. An inadequate counter-expression of TIMP-1 may represent a crucial event for the development and perpetuation of tissue damage. (J Rheumatol 2002;29:826–31)

Key Indexing Terms:
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In idiopathic inflammatory arthritides, proliferating synovial membrane plays a pivotal role in cartilage and bone destruction[1]. In these disorders, macrophages and fibroblast-like cells are the major component of the lining layer of the synovial tissue, especially at the level of the cartilage-pannus junction. Both cell types produce proinflammatory cytokines, growth factors, chemokines, and degrading enzymes, all of which cause and perpetuate tissue damage[2-4].

The role played by proteolytic enzymes, such as collagenases, in the pathogenesis of rheumatoid synovitis was described over 30 years ago[5,6]. Matrix metalloproteinases (MMP) are a large family of proteolytic enzymes acting in both physiological conditions (e.g., embryonic development, organ morphogenesis, angiogenesis) and pathological conditions (e.g., chronic inflammatory diseases, tumors)[7].

In adult rheumatoid arthritis (RA), MMP are thought to play a pivotal role in matrix remodeling and cartilage and bone destruction. Overexpression of a number of MMP (mainly MMP-3 and MMP-1) has been reported both at the level of lining and sublining layers of rheumatoid synovial tissue[8-14] and in biological fluids, such as plasma and synovial fluid (SF)[15-18].

Together with other proinflammatory cytokines and growth factors, tumor necrosis factor (TNF)-α plays an important role in the regulation of the expression of many MMP by synovial synoviocytes[19].

Four different natural inhibitors of MMP, namely, tissue inhibitor of metalloproteinase-1 (TIMP-1 to TIMP-4), interact with activated MMP, forming a 1:1 stoichiometric complex[7]. TIMP-1 is the most abundantly expressed natural MMP inhibitor in the lining cells of adult rheumatoid synovium[10-12,20,21] and has also been detected in both sera and SF from patients with RA[14-18,22].

We investigated the serum and SF concentrations of MMP-3 and TIMP-1 in juvenile idiopathic arthritis (JIA) and their relationships with TNF-α involvement.

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MATERIALS AND METHODS

A total of 273 patients with JIA according to the ILAR Durban criteria[23] have been evaluated during the last 5 years at the Rheumatology Unit of the G. Gaslini Institute. A serum sample is collected with permission at each control visit or at the time of hospitalization for a disease flare, and is immediately centrifuged and stored at –80˚ to avoid possible bias due to differences in clotting, processing, and storage induced alterations among the various samples. Moreover some clinical variables (number of active joints and joints with limited range of motion, and physician global assessment of overall disease activity) and laboratory (erythrocyte sedimentation rate, ESR) variables of disease activity are concomitantly recorded together with the ongoing treatment[24].

A retrospective analysis of case histories was performed to select sera from JIA patients with active and inactive disease. Disease activity was defined according to: (1) presence of active arthritis (swelling, or if absent, limitation of motion or tenderness) at least in one joint at the time of clinical examination[24]; (2) physician global estimate of disease activity > 5 (measured on a 0–10 cm visual analog scale)[25]; (3) ESR > 20 mm/h (for polyarticular course only). The disease was defined as inactive when (1) no history of arthritis or arthralgia 2 weeks before and absence of any sign of active arthritis (as defined above) at the time of clinical examination was recorded; (2) the physician global estimate of disease activity was = 0; (3) ESR was < 20 mm/h (for polyarticular course only).

Forty-five patients with JIA were selected. At the time of their disease onset, 4 patients displayed a systemic form, 3 patients had a rheumatoid factor (RF) negative polyarticular form and 38 patients an oligoarticular form. During the course of their disease 15 patients had a polyarticular course, whereas 30 had a persistent oligoarticular course (see below). Moreover 15 patients with inactive JIA (all with oligoarticular onset and course of the disease) were also selected.

Fifteen age matched healthy subjects attending at our clinic for routine preoperative examinations (e.g., orchiopexy, phimosis correction) were used as controls after parents gave informed consent. History of inflammatory or infectious disorders in the 4 weeks before the examination, together with clinical or laboratory signs of inflammation at the time of the study (i.e., elevated ESR or C-reactive protein), were considered as criteria of exclusion.

A total of 75 sera samples were tested by commercial ELISA for MMP-3 (Amersham, Buckinghamshire, UK) and TIMP-1 (Amersham) according to manufacturer's instructions. Notably, assays both for MMP-3 and TIMP-1 detect bound and free MMP-3 and TIMP-1, respectively. Concomitant evaluation of p75 soluble TNF receptor (sTNFR; Medgenix, Fleurus, Belgium) was performed. From previous experience, we know this soluble receptor represents the most reliable indicator of TNF involvement, at least at the level of biological fluids[26-28].

The concentrations of MMP-3, TIMP-1, and p75 sTNFR were evaluated in paired SF samples from 19 patients with active JIA (6 with polyarticular and 13 with oligoarticular course) who underwent knee SF needle aspiration before local therapeutic infiltration with steroids. A previous steroid infiltration at the same joint in the last 6 months was considered an exclusion criterion.

Serum levels of MMP-3 and TIMP-1 were compared among 4 subgroups of patients (active JIA patients with polyarticular course, active JIA patients with oligoarticular course, inactive oligoarticular JIA patients, and healthy controls) using the nonparametrical Mann-Whitney U test. Correlations among all the variables considered were evaluated using the nonparametric Spearman rank test. A correlation was considered significant if $p < 0.005$ (Bonferroni correction for multiple comparisons). Differences between concomitant serum and SF determinations were evaluated by the Wilcoxon rank test. A multivariable analysis of variation (ANOVA) model correcting for disease activity (number of active joints, physician global estimate of disease activity, and ESR) was used to verify the possible influence of treatment on MMP-3 and TIMP-1 serum and SF concentrations (see below).

RESULTS

The clinical characteristics of selected patients with JIA at the time of study are reported in Table 1. A total of 45 sera obtained during the active phase of disease were evaluated. As stated, at the time of study, 15 patients displayed a polyarticular course (4 systemic onset, 3 RF negative polyarticular onset, 8 extended oligoarticular course), whereas 30 patients displayed a persistent oligoarticular course[23]. At the time of serum sampling 21/30 patients with persistent oligoarticular disease were positive for antinuclear antibodies. The ongoing treatment at the time of study is reported in Table 1. Among active patients, 16 were treated with nonsteroidal antiinflammatory drugs (NSAID) alone (Group A), 12 a combination of NSAID and methotrexate (MTX) (Group B), and 8 with NSAID and oral steroids (Group C).

Fifteen sera from patients with persistent oligoarticular course and clinical evidence of disease inactivity were also studied (Table 1). In these patients, the mean duration of disease inactivity at the time of study was 5.1 months (range 0.5–23).

MMP-3 serum concentrations were significantly higher in patients with active JIA with polyarticular course (median 213.2 ng/ml, range 18.2–490) and JIA with active oligoarticular course (64 ng/ml, 10.2–610) than in inactive oligoarticular JIA (17.6 ng/ml, 2.4–88; $p = 0.04$ and $p = 0.02$, respectively) or healthy controls (2.4 ng/ml, 0–46; $p < 0.001$ for both; Figure 1A).

TIMP-1 serum concentrations were significantly higher in patients with active polyarticular (median 898.7 ng/ml, range 802–943) and oligoarticular JIA (871 ng/ml, 616–979) than in healthy controls (453.2 ng/ml, 322–752; $p = 0.03$ and $p = 0.01$, respectively). Conversely, inactive oligoarticular JIA patients showed significantly higher TIMP-1 concentrations (948 ng/ml, 440–968) than patients with active polyarticular ($p = 0.01$) or oligoarticular JIA ($p = 0.004$) (Figure 1B).

Notably, within JIA patients with a polyarticular course, no differences were noted for MMP-3 and TIMP-1 concentrations among patients with systemic onset (median MMP-3: 158.4 ng/ml, range 18.8–469; median TIMP-1: 887.2 ng/ml, 856–906), RF negative polyarticular onset (MMP-3: 253 ng/ml, 190–236; TIMP-1: 900 ng/ml, 896–908), and extended oligoarticular form (MMP-3: 215 ng/ml, 41–425; TIMP-1: 852 ng/ml, 748–911).

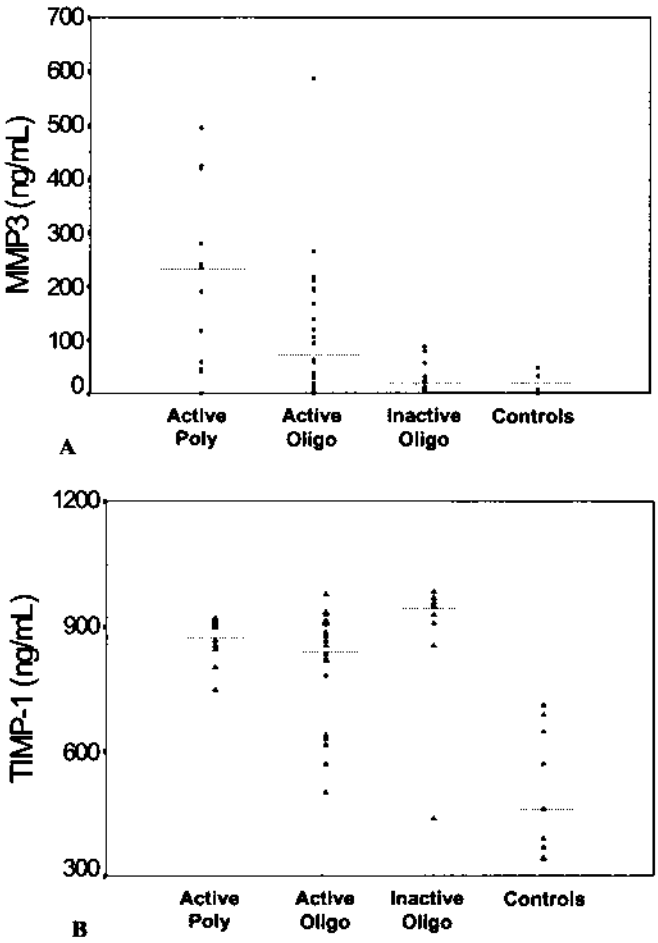
When the behavior of the serum MMP-3/TIMP-1 ratio in the 4 subgroups was considered, a significantly higher ratio was found in active polyarticular JIA patients than in inactive oligoarticular JIA patients ($p = 0.03$) and healthy controls ($p = 0.02$). Conversely, no statistically significant difference was observed between active oligoarticular patients and inactive oligoarticular patients ($p = 0.24$) or healthy controls ($p = 0.18$) (Figure 2A).

SF MMP-3 concentrations (median 4868 ng/ml, range

Table 1. Clinical and laboratory features of patients with JIA at the time of study.

	Age, mean (range), yrs	Disease duration, yrs	No. of joints Active/Limited ROM	PGI	ESR	Treatment [pts]
Active						
Polyarticular course [15pts]	7.2 (3.3–16.1)	3.7 (0.5–11.2)	7/12.2 (1–16)/(1–29)	8.8 (7–10)	78 (24–137)	NSAID, MTX [8] NSAID, CS, MTX [3] NSAID, CS [2] NSAID alone [2]
Oligoarticular course [30 pts]	8.5 (3–17.9)	2.4 (0.3–14)	1.3/1.5 (1–3)/(1–3)	6.9 (6–10)	35.5 (7–104)	NSAID alone [14] NSAID, CS [6] NSAID, MTX [4] Nil [6]
Inactive						
Oligoarticular course [15 pts]	7.3 (3.8–15)	3.4 (0.9–15)	0/0	0	13.5 (2–20)	NSAID alone [6] MTX, NSAID [1] Nil [8]

ROM: range of motion; PGI: physician global index; ESR: erythrocyte sedimentation rate; NSAID: nonsteroidal antiinflammatory drugs; MTX: methotrexate; CS: corticosteroids.



3972–4964.4) were 3–300 times higher than those detected in paired sera (median 213 ng/ml, range 10.1–1350; $p < 0.001$, Wilcoxon rank test). Moreover, SF TIMP-1 concentrations (917 ng/ml, 886.4–980 ng/ml) were significantly higher than those detected in paired serum samples (775 ng/ml, 569.6–928; $p = 0.02$) (data not shown). Notably, a clear inversion of the MMP-3/TIMP-1 ratio was observed when sera ratios (median 0.31, 0.02–1.5) were compared with the correspondent SF values (5.3, range 4.9–5.5; $p < 0.001$, Wilcoxon rank test; Figure 2B).

Taking into account the difference in the molecular weight between MMP-3 (57 kDa) and TIMP-1 (28 kDa), serum and SF concentrations were reconverted to be expressed as μmol/ml. Median MMP-3 molar concentrations were 85.4 μmol/ml (range 69.6–87) in SF and 3.7 μmol/ml (range 0.1–23.6) in paired sera. Median TIMP-1 SF molar concentration was 32.1 μmol/ml (range 31.1–34.3), whereas corresponding serum concentrations were 27.1 μmol/ml (range 19.9–32.5) (data not shown). Thus in SF, MMP-3 displayed a 2.6-fold molar excess versus corresponding TIMP-1. Conversely, in sera, TIMP-1 displayed a 7.3-fold molar excess versus MMP-3.

MMP-3 serum concentrations displayed a significant

Figure 1. Differences in MMP-3 (A) and TIMP-1 (B) serum concentrations among the 3 subgroups of JIA patients and healthy controls. Horizontal lines represent median values in each study group. A. MMP-3 serum concentrations were significantly higher in active polyarticular and active oligoarticular patients than in inactive oligoarticular patients ($p = 0.04$ and $p = 0.02$, respectively) or healthy controls ($p < 0.001$ for both). B. TIMP-1 serum concentrations were significantly higher in active polyarticular and oligoarticular patients than in healthy controls ($p = 0.03$ and $p = 0.01$, respectively). Conversely, inactive oligoarticular patients showed higher TIMP-1 concentrations than active polyarticular ($p = 0.01$) or oligoarticular ($p = 0.004$) patients.

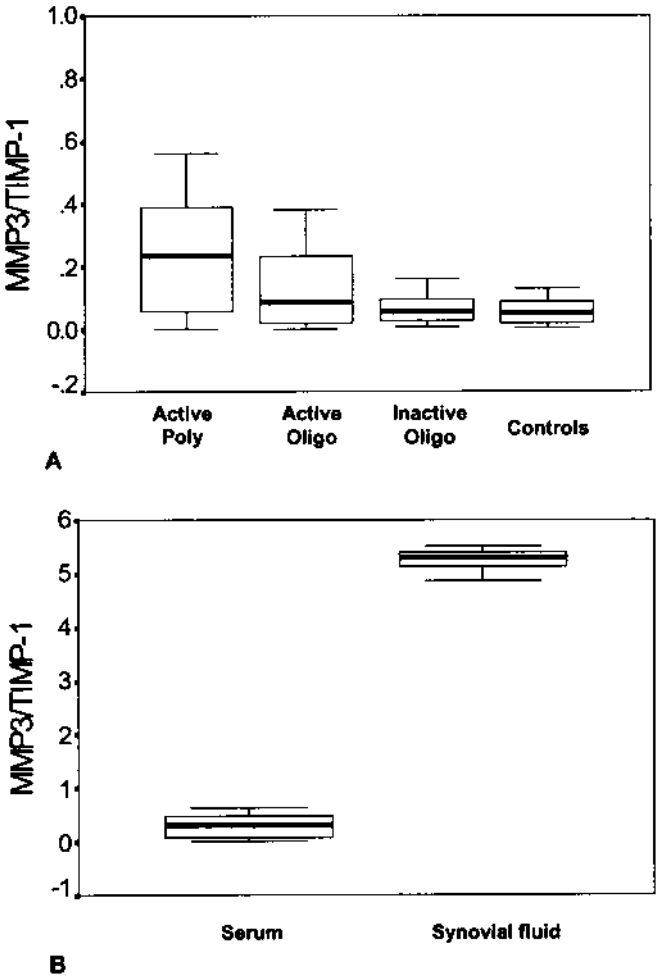


Figure 2. A. Serum MMP-3/TIMP-1 ratio in the 4 subgroups of patients studied. A significantly higher ratio was found in active polyarticular JIA than in inactive oligoarticular JIA ($p = 0.03$) or healthy controls ($p = 0.02$). B. Differences in MMP-3/TIMP-1 ratio between paired serum and SF samples. A clear inversion of MMP-3/TIMP-1 ratio was observed ($p < 0.001$, Wilcoxon rank test). Bold horizontal lines represent median values; boxes contain the 50% of values falling between the 25th and 75th percentiles, whisker lines that extend from the boxes represent the highest and lowest values for each subgroup.

correlation with a number of clinical and laboratory disease activity variables and with p75 sTNFR (Table 2), whereas TIMP-1 serum concentration did not (Table 2). No significant correlation was found between MMP-3 and TIMP-1 serum and SF concentrations. In SF, both MMP-3 and TIMP-1 showed a clear correlation with p75 sTNFR ($r = 0.43$, $p = 0.04$ and $r = 0.61$, $p = 0.005$, respectively; data not shown).

The possible influence of MTX or steroid treatment on MMP-3 and TIMP-1 serum concentration[29] was evaluated using a multivariable ANOVA model correcting for disease activity (number of active joints, physician global estimate of disease activity, ESR). No differences among Group A (NSAID treatment alone), Group B (NSAID plus MTX), or Group C (NSAID plus corticosteroids) were noted (data not shown).

Table 2. Correlation of MMP-3 and TIMP-1 serum concentration and clinical and laboratory variables of disease activity.

Variables	MMP-3	TIMP-1	p75 sTNFR	ESR
Clinical				
No active joints	r = 0.57	r = 0.12	r = 0.32	r = 0.73
	p = 0.0001*	p = 0.4	p = 0.02	p = 0.0001*
PGI	r = 0.50	r = 0.09	r = 0.33	r = 0.70
	p = 0.0001*	p = 0.6	p = 0.01	p = 0.0001*
Laboratory				
p75 sTNFR	r = 0.37	r = 0.18	—	r = 0.45
	p = 0.005*	p = 0.32	—	p = 0.001*
ESR	r = 0.50	r = 0.06	r = 0.45	—
	p = 0.0001*	p = 0.7	p = 0.001*	—

* Statistically significant according to Bonferroni correction for multiple comparisons ($p < 0.005$).
PGI: physician global index; sTNFR: soluble tumor necrosis factor receptor; ESR: erythrocyte sedimentation rate.

DISCUSSION

MMP are a large family of zinc dependent endoproteases that degrade most extracellular matrix components. All MMP are synthesized as proenzymes by a wide range of cell types. Their expression is transcriptionally regulated by different growth factors (e.g., platelet derived growth factor, fibroblast growth factor), proinflammatory cytokines (mainly TNF-α and interleukin 1), and hormones (insulin-like growth factor I, prolactin). Inactive pro-MMP are secreted and subsequently activated *in vivo* by tissue or plasma proteinases through the proteolytic cleavage of the propeptide domain at the N-terminus of the molecule[7].

The biological activity of all MMP is inhibited *in vivo* by 4 specific endogenous tissue inhibitors called TIMP. TIMP control connective tissue breakdown both by blocking the action of the activated MMP and by preventing their activation[7]. In physiological conditions, TIMP levels exceed those of active MMP. *In vitro* and *in vivo* studies have shown that TIMP can also inhibit cell invasion, tumorigenesis, and neoangiogenesis mediated by MMP[30]. TIMP are expressed by the same cells that produce MMP, mainly upon stimulation with proinflammatory cytokines[7,19].

To our knowledge, no information is yet available on MMP and TIMP concentrations in SF of patients with JIA. In this study, the concomitant evaluation of MMP-3 in sera and SF showed a clear overexpression of this protease at the site of chronic inflammation, with SF concentrations 3 to 300 times higher than in paired serum samples. Further, taking into account the clear molar excess of MMP-3 versus corresponding TIMP-1, it is possible that there is inadequate SF production of this major MMP natural inhibitor. It is notable that TIMP bind to MMP to form a tight ($K_i < 1$ nm) 1:1 stoichiometric noncovalent complex[31].

Although MMP-3 and TIMP-1 mRNA coexpression was detected in intimal lining cells from RA patients[12], impaired TIMP-1 protein production has been reported in studies of

RA, at the level of both SF and tissue[11,17,32,33]. This latter issue has been recently confirmed also on synovial tissue of patients with JIA (Gattorno, *et al*, unpublished results).

According to Burger and coworkers, a possible explanation for these observations may be found in a different modulation of MMP and TIMP production by synoviocytes after cell–cell contact with activated T lymphocytes[34].

MMP-3 serum concentrations were found to be significantly higher in patients with active versus those with inactive JIA, or in healthy controls. These findings are in accord with conclusions of studies in RA[16-18] and a serological study in JIA[35]. Moreover, serum MMP-3 were found to positively correlate with a number of clinical and laboratory variables of disease activity[36,37].

The investigation of the influence of treatment on MMP production was not a goal of the present study. Due to the wide range of disease subtypes and treatments this question was particularly difficult to investigate in a retrospective study. However, the use of a multivariate analysis correcting for the degree of disease activity allowed us to grossly exclude the presence of a clear influence of treatment on MMP-3 and TIMP-1 production, as shown in RA for MTX[29]. Notably, MMP serum levels may also be significantly influenced by individual patient physical activity before sampling[38]. This particular issue may explain the relative degree of variability of MMP-3 serum concentration we found in patients with active JIA.

In relatively recent inactive patients with the persistent oligoarticular form, serum TIMP-1 concentrations were significantly higher than in active persistent oligoarticular patients. This finding is consistent with a previous observation in RA patients showing significantly higher TIMP-1 concentrations in patients with mild or moderate functional disability than in patients with severe disability[39].

An additional feature coming from the study concerns the possible functional interaction between MMP-3 and TIMP-1 and proinflammatory cytokines, such as TNF-α. Together with other proinflammatory cytokines and growth factors, TNF-α plays an important role in regulation of the expression of both MMP and TIMP[19,40]. Conversely, the proteolytic activities of some MMP (mainly MMP-3 and MMP-13) have been shown to be crucial for the functional activation of proinflammatory cytokines. TNF-α, for example, is initially expressed as a 233 amino acid membrane anchored precursor that is proteolytically processed by MMP to yield the functional 157 amino acid cytokine[41]. In this study, a significant and strong correlation between sTNFp75 and MMP-3 levels was found, suggesting the existence of mutual functional relationships between TNF and MMP. Interestingly, synthetic MMP inhibitors can decrease *in vitro* membrane TNF-α release by many cell types[42].

These observations may provide additional support to the development of an anti-MMP strategy for treatment of idiopathic inflammatory arthritides[43,44].

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